PATENT
09/994,440
Docket 091/010c

AMENDMENTS TO SPECIFICATION

Please change the TITLE of the application as follows:

~~**Techniques for Growth and Differentiation of Human Plurlpotent Stem Cells**~~

Culturing and Differentiating Human Embryonic Stem Cells

The following amendments are made with reference to this application as published: US-2002-0090723-A1

Please AMEND paragraph [0001] of the published application as follows:

[0001] This application is a continuation of U.S. Ser. No. 09/859,291, filed May 16, 2001 (pending), ~~and claims priority to the following patent applications: U.S. Ser. No. 60/175,581, filed Jan. 11, 2000; U.S. Ser. No. 60/213,740, filed Jun. 22, 2000; U.S. Ser No. 60/213,739, filed Jun. 22, 2000; U.S. Ser. No. 60/216,387, filed Jul. 7, 2000; U.S. Ser. No. 60/220,064, filed Jul. 21, 2000; U.S. Ser. No. 09/688,031, filed Oct. 10, 2000; and~~ which is a continuation of PCT/US01/01030, (designating the U.S.) filed Jan. 10, 2001, and published as WO 01/51616 on Jul. 19, 2001 ; through which it claims priority to provisional applications U.S. Ser. No. 60/175,581, filed Jan. 11, 2000; U.S. Ser. No. 60/213,740, filed Jun. 22, 2000; U.S. Ser No. 60/213,739, filed Jun. 22, 2000; U.S. Ser. No. 60/216,387, filed Jul. 7, 2000; U.S. Ser. No. 60/220,064, filed Jul. 21, 2000; and U.S. Ser. No. 09/688,031, filed Oct. 10, 2000 (now U.S. Patent No. 6,667,176).

Please AMEND paragraph [0103] of the published application as follows:

[0103] pPS cells plated in the absence of fresh feeder cells benefit from being cultured in a nutrient medium. The medium will generally contain the usual components to enhance cell survival, including isotonic buffer, essential minerals, and either serum or a serum replacement of some kind. ~~Particularly beneficial is a medium that has been conditioned to supply some of the elements provided by feeder cells.~~ The medium can be conditioned by culturing with another cell population, or it can comprise a synthetic mixture of factors that promote growth of the hPS cells while inhibiting differentiation.

Please cancel or disregard the amendment to the specification made in the paper filed May 14, 2004.

Instead, please AMEND paragraphs [0106] and [0107] of the published application as follows:

[0106] If desired, conditioned medium can be supplemented before use with additional growth factors that benefit pPS cell culture. For hES, a growth factor like bFGF or FGF-4 is often used. For hEG, culture medium may be supplemented with a growth factor like bFGF, an inducer of gp130, such as LIF or Oncostatin-M, and perhaps a factor that elevates cyclic AMP levels, such as forskolin. Other types of pPS cells may benefit from other factors in the medium, such as stem cell factor (Steel factor, c-kit ligand), or IL-6. It is often beneficial to add growth factors such as bFGF or FGF-4 to the medium both before conditioning, and then again before using the medium to support the growth of pPS cells. It is recognized that the beneficial effects of fibroblast conditioned medium are derived from soluble factors produced by the embryonic fibroblasts, and that synthetic mixtures having similar components in various combinations may also be beneficial.

[0107] It should be recognized that each of the conditions described here can be optimized independently, and certain combinations of conditions will prove effective upon further testing. Such optimization is a matter of routine experimentation, and does not depart from the spirit of the invention provided in this disclosure.

Please AMEND paragraph [0184] of the published application as follows:

[0184] The sequence data provides a general estimate of the diversity of the CDNA library, based on the number of independent genes represented. For instance, comparing the cDNA sequences to the UNIGENE collection (available at the website of the National Center for Biotechnology Information ~~; http://www.ncbi.nlm.nih.gov/UniGene/index.html~~) allows assignment of a unique cluster identifier for most sequences. By comparing the number of assigned cluster identifiers to the total number of cDNAs evaluated, an estimate of the clone diversity can be achieved.

Please AMEND paragraph [0232] of the published application as follows:

[0232] The use of microarray in analyzing gene expression is reviewed generally by Fritz et al Science 288:316, 2000; "Microarray Biochip Technology", M. Schena ed., Eaton Publishing Company; "Microarray analysis", Gwynne & Page, Science (Aug. 6, 1999 supplement); Pollack et al., Nat Genet 23:41, 1999; Gerhold et al., Trends Biochem. Scl. 24:168, 1999; "Gene Chips (DNA Microarrays)", ~~L. Shi at the Internet URL www.Gene-Chips.com~~ from the website by Leming Shi, Ph.D. Systems and reagents for performing microarray analysis are available commercially from companies such as Affymetrix, Inc., Santa Clara Calif.; Gene Logic Inc., Columbia Md.; HySeq Inc., Sunnyvale Calif.; Molecular Dynamics Inc., Sunnyvale Calif.; Nanogen, San Diego Calif.; and Synteni Inc., Fremont Calif. (acquired by Incyte Genomics, Palo Alto Calif.).